A LIGHT AND ELECTRON MICROSCOPE SURVEY OF ALGAL CELL WALLS. II. CHLOROPHYCEAE[1]

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ABSTRACT

Under the light and electron microscopes, the structure of the cell walls of members of the 11 orders in the Class Chlorophyceae, Division Chlorophyta, were examined. With regard to the microfibrillar component of the cell walls, five types of wall structure were distinguished: (A) an apparent lack of a microfibrillar component (Volvocales, Dasycladales, and some members of the Siphonales), (B) the microfibrils are arranged in a reticulate pattern (Tetrasporales, Schizogoniales), (C) the microfibrils are oriented in an axial direction (Ulotrichales, Oedogoniales, Zygnematales, and some members of the Siphonales), (D) the microfibrils are parallel to one another and arranged in lamellae (Ulvales), and (E) the microfibrils are parallel to one another, arranged in lamellae, and at right angles to the microfibrils in the lamellae above and below forming the cross-fibrillar pattern (Cladophorales, Siphonocladales). Members of the Ulvales were found to have a cell wall similar to that of the brown algae while a member of the Schizogoniales, *Prasiola*, was found to have a cell wall similar to that of the red algae. A discussion of the taxonomic implications of cell wall structure is included.

The Division Chlorophyta, as delimited by Smith (1955), includes two classes, the Chlorophyceae, with the majority of green algae from unicellular to filamentous and complex parenchymatous thalli, and the Charophyceae, with only one order. The evolution within this Division is of especial interest because of its presumed close relationship with extant land plants (Fritsch, 1935). Similarities between the Chlorophyta and land plants include (1) chromatophores with similar chlorophyll and carotinoid pigments; (2) starch as a storage product; (3) the structure and insertion of flagella; and (4) the presumed possession of walls containing cellulose.

A considerable amount is known of the structure and chemical nature of certain algal cell walls, but in the class Chlorophyceae most of the previous work has been concentrated on members of two orders, the Cladophorales and Siphonocladales. In both orders, the cell wall consists of highly crystalline cellulose microfibrils arranged parallel to one another in lamellae. These lamellae are oriented at right angles to each other with a third, less common direction, sometimes bisecting the first two (*Valonia*, Cronshaw and Preston, 1958; *Dictyosphaeria*, Steward and Muhlethaler, 1953; *Cladophora* and *Chaetomorpha*, Frei and Preston, 1961a). Two members of the Cladophorales appear to lack a microfibrillar component in the cell wall. Instead, the wall is amorphous in nature (*Spongomorpha* and *Acrosiphonia*, Nicolai and Preston, 1959).

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In other orders of the Chlorophyceae, evidence for a microfibrillar component in the cell wall has been found in *Chlamydomonas* (Sager and Palade, 1957); *Spirogyra* (a multinet growth pattern, Limaye et al., 1963); *Ulva* and *Enteromorpha* (a reticulum of microfibrils, Cronshaw et al., 1958); *Chlorella* (a reticulum of microfibrils, Northcote et al., 1958); *Hydrodictyon* (lamellae of microfibrils, Northcote et al., 1960); *Oedogonium* (lamellae of microfibrils, Parker, 1965); *Halicystis* (a polylamellar reticulum of xyloglucan microfibrils, Roelofsen et al., 1953); *Bryopsis, Caulerpa, Penicillus, Udotea,* and *Halimedia* (an axial orientation of 1–3 xylan microfibrils, Frei and Preston, 1961b); and *Dichotomosiphon* (1–3 xylan component, Parker et al., 1963). Cell walls lacking a microfibrillar component have been described for *Chlamydomonas* (Lewin et al., 1951); members of the Volvocaceae and Astrephemonaceae (Lang, 1963; Parker, 1965); *Pediastrum* (the cell wall consists of hexagonal units of an unknown substance, Moner and Chapman, 1963; which is not cellulose but consists of chains of rings, Parker, 1965); *Codium, Dasycladus, Acetabularia,* and *Batophora* (an amorphous wall structure composed of mannan polymers, Frei and Preston, 1961b).

This preliminary survey is a light- and electron-microscope study of the Chlorophycean cell wall structure, with special regard to the microfibrillar component. In order to cover as many representatives as possible in determining the usefulness of the green algal cell wall as a taxonomic character, a review of previous work is included. Although the microfibrillar component may be but a small portion of the total cell wall, microfibrils can be set off from other wall substances as the primary framework because of the unusual size and high degree of molecular order. Consistent patterns within a taxa might then indicate relationships. The Charophyceae are not included since they have been treated comprehensively by Green (1960). Detailed studies of the chemical composition of the apparently amorphous walls and the interfibrillar substances which do not mask the fundamental pattern of the wall of specific forms will be considered in future work.

MATERIALS AND METHODS

Specimens of the green algae were collected in tide pools and by skin diving on the coasts of southern California, southern Florida, Puerto Rico, and southern Victoria, Australia. Fresh-water specimens were collected near the Mountain Lake Biological Station, Pembroke, Virginia, and in various mountain streams in southern California. The material was carried alive in polyethylene bags to the laboratory for study or was fixed immediately in 2 per cent formalin.

The validity of the microchemical stains used, the methods of removal of incrusting substances, and the details of maceration including ultrasonic fragmentation have been previously discussed (Dawes et al., 1961). The cell wall of members of the Cladophorales and Siphonocladales can be separated into individual lamellae by removal of pectic material if the filament is bathed for 20 min in 2 per cent NH_4OH at 60°C.

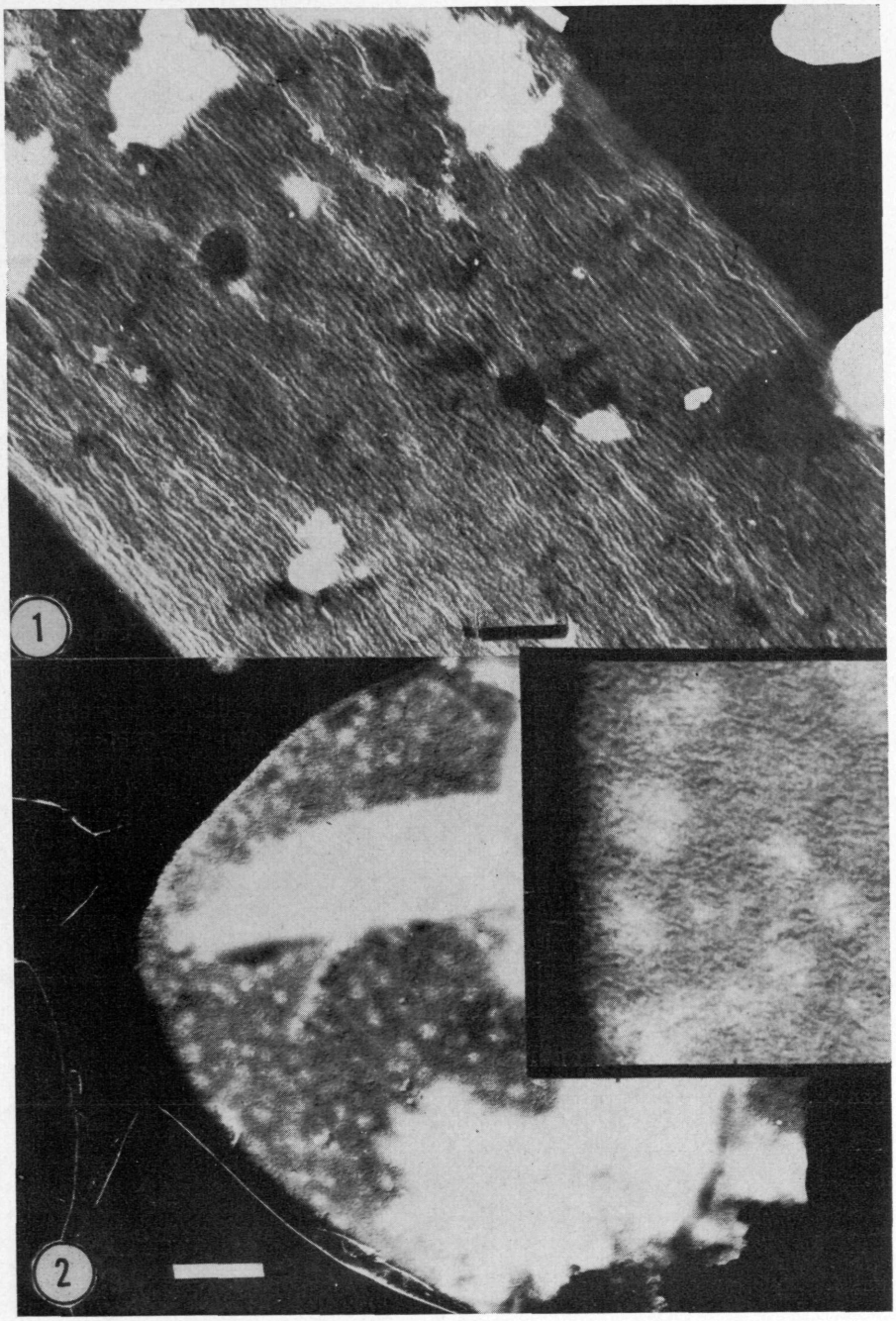
Transverse, ultrathin sections (fig. 6a, 6b) were obtained using a Si-Ro-Flex

EXPLANATION OF FIGURES

All unit marks equal one micron.

FIGURE 1. *Acetabularia crenulata.* The chemically cleared, ultrasonically macerated filament wall consists of an amorphous matrix, which is finely striated possibly due to folding. ×9,600.

FIGURE 2. *Tetraspora* sp. A single cell isolated from the colonial matrix by ultrasonic maceration has a cell wall composed of a microfibrillar reticulum. The opaque structure is due to folding of the wall after drying. ×10,000. The insert is a portion of *Tetraspora* wall showing detail of microfibrillar "weft". ×30,000.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ultramicrotome after fixation in unbuffered 2 per cent $KMnO_4$ for 15 min at 0°C, in sea water (pH 6.7), dehydration in 10 per cent steps of butyl alcohol for 1 hr at each step, and embedding in methyl methacrylate. The sections were then washed twice in chloroform to remove the methacrylate and shadowed with platinum. The carbon replica of the interior wall of *Apjohnia* (fig. 7) was prepared by the wet replica technique (Wardrop, 1964).

OBSERVATIONS AND DISCUSSIONS

The following species were examined under the light and electron microscopes in the present study.

Chlamydomonas reinhardi
Haematococcus australis
Volvox sp.
Tetraspora sp.
Stigeoclonium sp.
Ulothrix sp.
Spirogyra sp.
Chaetomorpha aerea
C. torta
C. Darwinii
Cladophora trichotoma
C. gramineae
C. microcladioides
C. sp. (fresh water)
Rhizoclonium sp.
Urospora penicilliformis
Anadyomene stellata
Spongomorpha coalita
Ulva taeniata
Entermorpha sp.
Prasiola meridionales
Chlorella sp.
Codium fragile
Bryopsis pennata
Cladophorophsis membranacea
Apjohnia laetevirens
Struvea plumosa
Dasycladus vermicularis
Acetabularia crenulata
Batophora oerstedi

On the basis of their known cell-wall structure, each of these green algae can be placed into one of five categories according to the presence of a microfibrillar component where none was previously reported.

Group A.—The cell walls appear to lack a microfibrillar component. The representatives examined include unicellular, colonial, and siphonaceous forms from the orders Volvocales (*Chlamydomonas*, *Haematococcus*, *Volvox*), Siphonales (*Codium*), (Cladophorales (*Spongomorpha*), and Dasycladales (*Acetabularia*, *Batophora*, *Dasycladus*). Under the light microscope, with microchemical stains, the cell wall gives a positive reaction for pectin substances (ruthenium red) and a positive reaction for cellulose (I_2KI, H_2SO_4) with the exception of *Spongomorpha*, *Codium*, *Dasycladus*, *Batophora*, and *Acetabularia*.

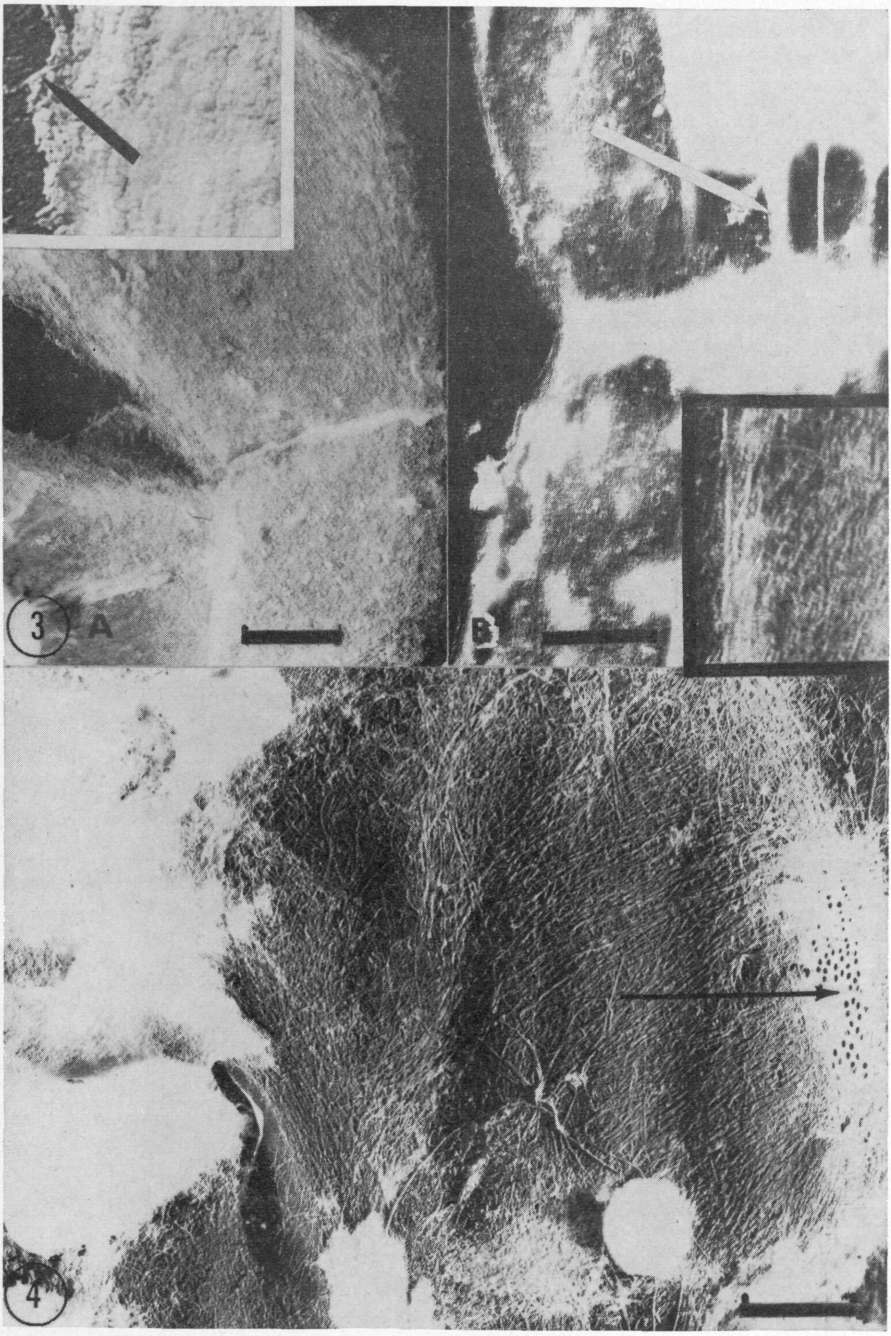
Under the electron microscope, the cell wall of the members examined is granular in appearance and striations or folds are present (*Acetabularia* fig. 1). Regardless of chemical treatment used to remove possible incrusting substances,

EXPLANATION OF FIGURES

FIGURE 3A. *Prasiola meridionalis*. The chemically cleared and ultrasonically macerated cell wall of three cells consists of a reticulum of microfibrils with an impregnating amorphous matrix. ×10,800. The insert is a portion of *Prasiola* wall, showing microfibrils (arrow) and the covering amorphous matrix. ×32,400.

FIGURE 3B. *Stigeoclonium* sp. The chemically cleared cell wall of the filament consists of microfibrils arranged in an axial direction in the older, elongated cells. Plasmodesmata are visible transversing the end walls and connecting adjacent protoplasts. ×11,200. The insert is a portion of *Stigeoclonium* wall showing axially directed microfibrils. ×33,600.

FIGURE 4. *Enteromorpha* sp. A cell wall with parallel microfibrils in lamellae, of a portion of three cuboidal cells of the chemically cleared and ultrasonically macerated thallus is visible. A pit composed of a group of pores with abundant amorphous material covering the microfibrils is present on one side of the cell wall (arrow). ×12,000.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no fibrillar material is found. The results of Frei and Preston (1961b) on the cellular wall structure of *Dasycladus*, *Batophora*, and *Acetabularia* (Dasycladales) is in agreement with the present study. Studies on *Pediastrum* (Moner and Chapman, 1963; Parker, 1964) indicate that it also lacks a microfibrillar component and should be placed in this group. However, microfibrils are present in other members of the Siphonales (Frei and Preston, 1961b; Parker et al., 1963) and Chloroccales (Northcote et al., 1960), indicating probable taxonomic divergences within the orders.

Group B.—The cell wall contains a microfibrillar component in a reticulate pattern. Under the light microscope, with microchemical stains, the cell walls of *Tetraspora* (Tetrasporales, colonies of spherical cells embedded in a matrix) and *Prasiola* (Schizogoniales, a parenchymatous thallus) give a positive reaction for cellulose and pectic substances.

Under the electron microscope, the loose weft arrangement of microfibrils in the cells is present in *Tetraspora* (fig. 2) and *Prasiola* (fig. 3a). No change in orientation is visible in the older cell walls of the thallus of *Prasiola*. In the cell wall of *Tetraspora*, small white spots, possibly remaining protoplasm, are visible (fig. 2) when no chemical clearing is undertaken. The colonial matrix of *Tetraspora* is amorphous in nature. *Chlorella pyrenoidosa* (Chlorococcales) has been shown to possess a cell wall composed of microfibrils in a reticulate pattern (Northcote et al., 1958) and therefore falls into this group.

Group C.—The cell wall is constructed of microfibrils arranged in an axial direction. Under the light microscope, and with microchemical stains, the cell wall of the filamentous representatives of the Zygnematales (*Spirogyra*) and Ulothrichales (*Stigeoclonium*, *Ulothrix*) gives a positive reaction for cellulose and pectic substances. Plasmodesmata are visible between proplasts of the plasmolyzed cells of the filaments. The mucilaginous sheath in *Spirogyra* gives a strong reaction for pectic substances.

Under the electron microscope, an axial orientation of microfibrils is visible either without chemical treatment in older, elongated cells of *Stigeoclonium* (fig. 3b) and *Ulothrix*, or after removal of a mucilaginous sheath from the filaments of *Spirogyra* (fig. 5b). A reticulate pattern of microfibrils is evident in the bulbous, tip-cells of filaments of *Stigeoclonium* and *Ulothrix* and in the mucilage and outermost layer of microfibrils in untreated filaments of *Spirogyra* (fig. 5a). In *Spirogyra*, this microfibrillar reticulum covers a layer of axial microfibrils (fig. 5a, 5b). In a third and innermost layer, microfibrillar bands occur in transverse orientation (fig. 5c). Such a difference in microfibrillar arrangement (see also Limaye et al., 1963) in the inner and outer surfaces of the cell wall is consistent with the multinet growth hypothesis (Houwink and Roelofsen, 1954), although it appears that the microfibrils are reoriented as groups and not individually (in *Spirogyra*). Some members of the Siphonales (*Bryopsis*, *Caulerpa*, *Penicillus*, *Udotea*, *Halimeda*, Frei and Preston, 1961b), and possibly one member of the Chlorococcales, (*Hydrodictyon*, Northcote et al., 1960) possess microfibrils in axial orientation; however, the microfibrils consist of a xylan component.

Group D.—The cell walls consist of microfibrils parallel to one another and arranged in lamellae. Under the light microscope and with microchemical stains the parenchymatous thalli of *Ulva* and *Enteromorpha*, members of the Ulvales, give a positive reaction for pectic substances and cellulose.

As seen under the electron microscope after chemical clearing, the cell walls of both genera consist of parallel microfibrils in lamellae (*Enteromorpha*, fig. 4), which are arranged in flat spirals around the cell. Pits are either simple, thin areas in the cell wall, a random dispersion of pores, or groups of pores defined by an amorphous matrix (arrow, fig. 4). *Oedogonium* (Oedogoniales) has also been shown to possess lamellae of parallel microfibrils (Parker, 1964) and is placed here. This group appears to be intermediate between Group C and E in microfibrillar organization.

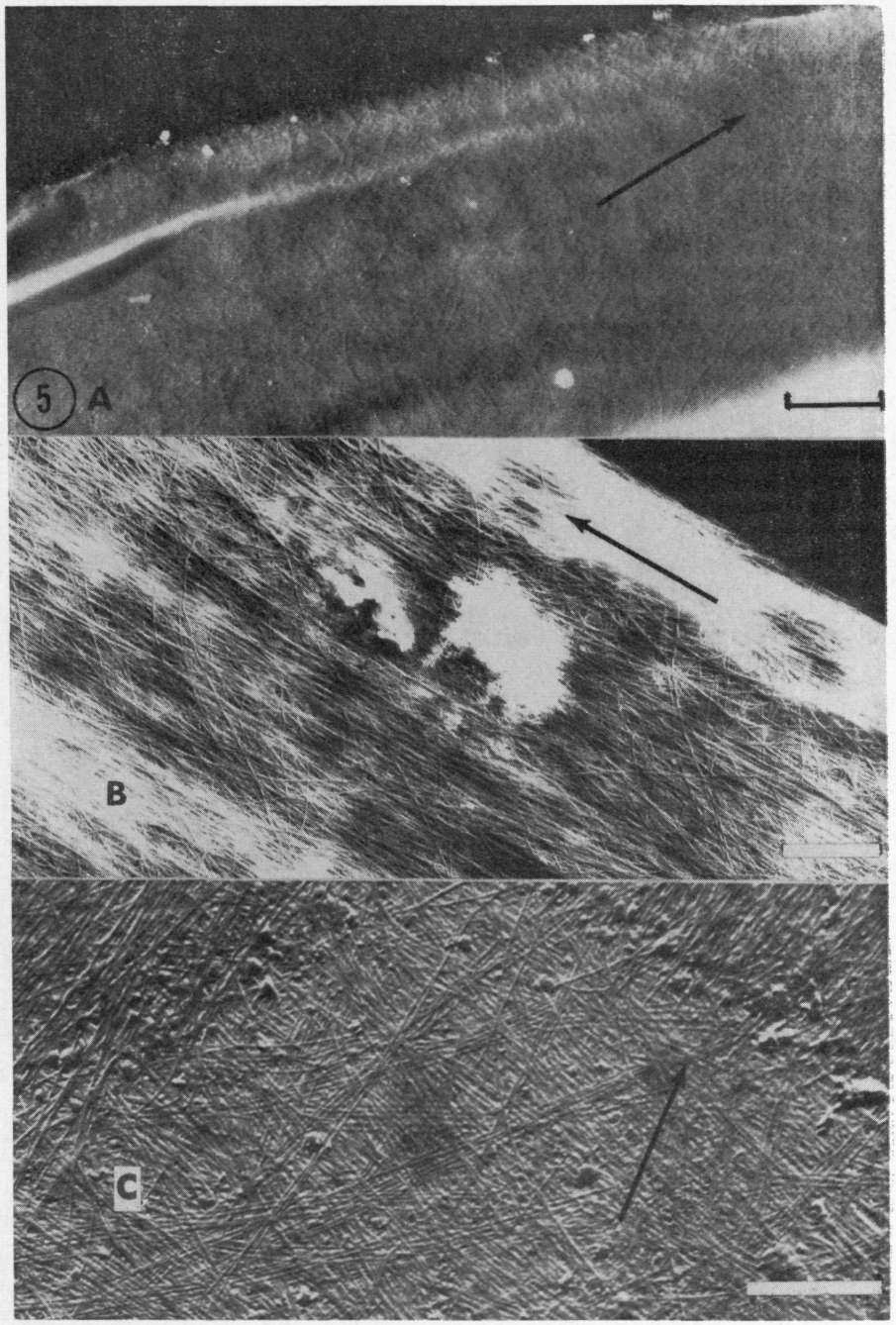


FIGURE 5. *Spirogyra* sp. The outer wall in untreated filaments consists of a reticulum of microfibrils and amorphous mucilage (fig. 5a). After chemical clearing, the outermost randomly arranged microfibrils are visible (fig. 5b). With maceration, an inner wall of transverse bands of microfibrils is visible (fig. 5c). Arrows indicate the filament's axis. Figures 5a and 5b, ×10,000; figure 5c, ×14,600.

Group E.—The cell walls consist of microfibrils oriented in a cross-fibrillar structure. Under the light microscope with microchemical stains, the thick stratified cell walls of members of the Cladophorales (*Cladophora, Chaetomorpha, Rhizoclonium, Urospora*) and members of the Siphonocladales (*Apjohnia, Struvea, Cladophoropsis,* and *Anadyomene*) all give positive reactions for pectic substances and cellulose. After chemical clearing, the lamellated cell wall of all the specimens, examined under the light microscope, shows two directions of striations, one transverse to the filament's axis in a flat spiral, the other parallel to the filament's axis in a steep spiral.

As seen under the electron microscope, the cell walls of *Apjohnia* (fig. 6a, 7) and *Chaetomorpha* (fig. 6b) have a thin outermost reticulate layer of microfibrils (*Chaetomorpha,* fig. 6b), which covers the filament and frays out onto the formvar. The remainder of the wall consists of lamellae of parallel microfibrils which are at right angles to the microfibrils in the lamellae above or below (*Apjohnia,* fig. 7), so that in cross section, the microfibrils of every third lamella are similar in orientation (*Apjohnia,* fig. 6a and *Chaetomorpha,* fig. 6b). Also, the inner lamellae are thicker than the outer ones (fig. 6a, 6b).

CONCLUSIONS

With present techniques for the determination of cell wall structure, and including previous studies, five types of cell wall patterns have been demonstrated among the members of the Chlorophyceae. This is in complete contrast to the uniform cell wall structure among the orders of the Phaeophyta or Rhodophyta. Furthermore, variations of cell wall chemistry and microfibrillar patterns, if present, are found within single orders (e.g. Siphonales and Chlorococcales). Such variations among and within orders of the Chlorophyceae lend credence to the general belief that this group is composed of widely diversified plants and that perhaps certain orders contain divergent groups (e.g. Siphonales).

Group E, however, includes members of the Cladophorales and Siphonocladales exclusively with a very distinct microfibrillar pattern (see also Cronshaw and Preston, 1958; Frei and Preston, 1961a). Fritsch (1946) discussed in detail the question of uniting the two orders and concluded that they show parallel evolution and not a phylogenetic relationship. The chemistry and microfibrillar pattern of the cell wall is common to both orders and distinct from other green algae (Nicolai and Preston, 1952), and may be used to support either the concept of parallel evolution or that of common phylogeny.

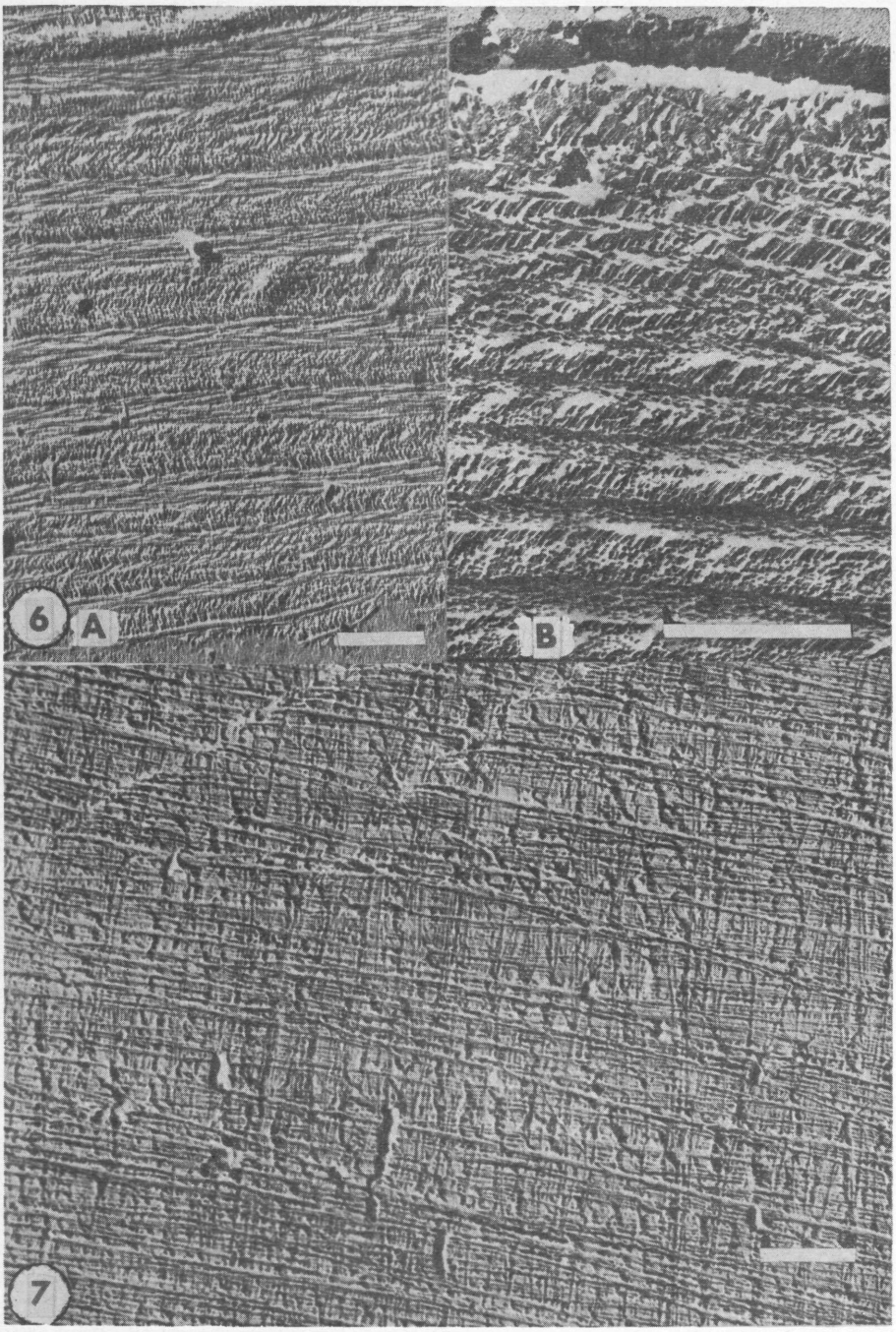
Members placed in Group B possess a cell wall with a reticulate microfibrillar framework resembling that of the red algal cell wall (Dawes et al., 1961). In addition, the chemistry of the cell wall has been found to be similar (Cronshaw et. al., 1958). A primary reticulate layer of microfibrils appears as the first wall in developing *Chaetomorpha* sporelings (Frei and Preston, 1961a) and, in the

EXPLANATION OF FIGURES

FIGURE 6a. *Apjohnia laetevirens.* A cross-section of the cell wall of a young filament treated as described in figure 6b. The orientations of microfibrils are (1) in a flat spiral around the filament (microfibrils parallel to the short edge of the plate) and (2) in a steep spiral around the filament (the microfibrils have fallen over after removal of the methacrylate). ×7,500.

FIGURE 6b. *Chaetomorpha Darwinii.* A slightly oblique section of the cell wall of a mature filament after methacrylate removal and metal shadowing showing the alternating lamellae of microfibrils oriented at right angles to one another. ×20,000.

FIGURE 7. *Apjohnia laetevirens.* A replica of the innermost layer of the untreated filament showing the typical crossed fibrillar structure with particles of protoplasm attached to some of the innermost microfibrils. ×7,300.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present survey, in all walls which possessed a fibrillar component (similar to land plant cell walls, Muhlethaler, 1961).

This survey is intended as an initial light- and electron-microscope study of the microfibrillar structure of green-algal cell walls with regard to taxonomic implications. More detailed studies of individual plants within these five groups are needed.

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REFERENCES

Cronshaw, J. and **R. D. Preston.** 1958. A re-examination of the fine structure of the walls of vesicles of the green algae *Valonia*. Proc. Roy. Soc. London B. 148: 137–148.

———, **A. Myers,** and **R. D. Preston.** 1958. A chemical and physical investigation of the cell walls of some marine algae. Biochem. Biophys. Acta. 27: 89–103.

Dawes, C. J., F. M. Scott, and **E. Bowler.** 1961. A light and electron microscope survey of algal cell walls. I. Phaeophyta and Rhodophyta. Amer. J. Bot. 48: 925–934.

Frei, E. and **R. D. Preston.** 1961a. Cell wall organization and cell wall growth in the filamentous green algae *Cladophora* and *Chaetomorpha*. I. The basic structure and its formation. Proc. Roy. Soc. London B. 154: 70–95.

———. 1961b. Variants in the structural polysaccharides of algal cell walls. Nature 192: 939–943.

Fritsch, F. E. 1935. Vol. I. The structure and reproduction of the algae. The University Press, Cambridge, England.

———. 1946. The status of the Siphonocladales. M. O. P. Iyengar Commemoration Volume. Ind. Bot. Soc.

Green, P. B. 1960. Multinet growth in the cell wall of *Nitella*. J. Biophys. Biochem. Cytol. 7: 289–296.

Houwink, A. L. and **P. A. Roelofsen.** 1954. Fibrillar architecture of growing plant cell walls. Acta. Bot. Neer. 3: 385–395.

Lewin, R. A., M. J. Owen, and **J. L. Melnick.** 1951. Cell wall structure in *Chlamydomonas*. Exptl. Cell. Res. 2: 708–710.

Limaye, V. J., P. A. Roelofsen, and **B. J. Spit.** 1963. Submicroscopic structure of the cellulose in the cell walls of *Spirogyra* and *Nitella*. Sci. Indus. 10: 8–20.

Moner, J. G. and **G. B. Chapman.** 1963. Cell wall formation in *Pediastrum biradiatum* as revealed by the electron microscope. Amer. J. Bot. 50: 992–998.

Nicolai, E. and **R. D. Preston.** 1959. Cell wall studies in the Chlorophyceae. III. Differences in structure and development in the Cladophoraceae. Proc. Roy. Soc. London B. 151: 244–255.

Northcote, D. H., K. J. Goulding and **R. W. Horne.** 1958. The chemical composition and structure of the cell wall of *Chlorella pyrenodosa*. Biochem. J. 70: 391–397.

———. 1960. The chemical composition and structure of the cell wall of *Hydrodictyon africanum* Yaman. Biochem. J. 70: 391–397.

Parker, B. C., R. D. Preston and **G. E. Fogg.** 1963. Studies of the structure and chemical composition of the cell walls of Vaucheriaceae and Saprolegniaceae. Proc. Roy. Soc. London B. 158: 435–445.

———. 1965. The structure and chemical composition of the cell walls of three Chlorophycean algae. Phycologia 4: 63–74.

Roelofsen, P. A., V. C. Dalitz and **C. R. Wijnman.** 1953. Constitution of the submicroscopic structure and degree of crystallinity of the cell wall of *Halicystis osterhautic*. Biochem. Biophys. Acta. 11: 344–352.

Sager, R. and **G. E. Palade.** 1957. Structure and development of the chloroplast in *Chlamydomonas*. I. The normal green cell. J. Biochem. Biophys. Cytol. 3: 463–488.

Smith, G. M. 1955. Cryptogamic botany. Vol. I. McGraw-Hill Book Co., New York.

Steward, F. C. and **K. Muhlenthaler.** 1953. The structure and development of the cell wall in the Valoniaceae as revealed by the electron microscope. Ann. Bot. 17: 295–316.

Wardrop, A. B. 1964. The structure and formation of the cell wall in xylem. p. 087–134. *In* Formation of wood in forest trees. M. L. Zimmerman, ed. Academic Press, New York.